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Genetic Relatedness of High-Level Aminoglycoside-Resistant Enterococci Isolated from Poultry Carcasses

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SUMMARY. Approximately 46% (75/162) or poultry enterococci collected between 1999 and 2000 exhibited high-level resistance to gentamicin (minimum inhibitory concentration [MIC] $\geq$ 500 μg/ml), kanamycin (MIC $\geq$ 500 μg/ml), or streptomycin (MIC $\geq$ 1000 μg/ml). Forty-one percent of the isolates were resistant to kanamycin ($n = 67$), whereas 23% and 19% were resistant to gentamicin ($n = 37$) and streptomycin ($n = 31$), respectively. The predominant species identified was *Enterococcus faecium* ($n = 105$), followed by *Enterococcus faecalis* ($n = 40$) and *Enterococcus durans* ($n = 8$). Using polymerase chain reaction, the isolates were examined for the presence of 10 aminoglycoside resistance genes [*ant(6)-Ia, ant(9)-Ia, ant(4′)-Ia, aph(3′)-IIIa, aph(2″)-Ib, aph(2″)-Ic, aph(2″)-Id, aac(6′)-Ie-aph(2″)-Ia,* and *aac(6′)-Ii*]. Five aminoglycoside resistance genes were detected, most frequently *aac(6′)-Ii* and *ant(6)-Ia* from *E. faecium*. Seven *E. faecalis* isolates resistant to gentamicin, kanamycin, or streptomycin were negative for all genes tested, indicating that additional resistance genes may exist. Phylogenetic analysis revealed that the isolates were genetically different with little clonality. These data indicate that enterococci from poultry are diverse and contain potentially unidentified aminoglycoside resistance genes.

RESUMEN. Correlación genética de cepas de enterococos que presentan niveles altos de resistencia contra aminoglicósidos, aislados a partir de canales de pollos.

Aproximadamente el 46% (75/162) de las cepas de enterococos aisladas en pollos en los años 1999 y 2000 presentaron niveles altos de resistencia contra gentamicina (dosis inhibitoria mínima $\geq$ 500 μg/ml), kanamicina (dosis inhibitoria mínima $\geq$ 500 μg/ml) o estreptomicina (dosis inhibitoria mínima $\geq$ 1000 μg/ml). Cuarenta por ciento de los aislados fueron resistentes a kanamicina ($n = 67$), mientras que 23% y 19% fueron resistentes a gentamicina ($n = 37$) y estreptomicina ($n = 105$), respectivamente. La especie predominante entre las cepas aisladas fue el *Enterococcus faecium* ($n = 105$), seguida por el *Enterococcus faecalis* ($n = 40$) y el *Enterococcus durans* ($n = 8$). Se examinaron las cepas aisladas mediante la prueba de reacción en cadena por la polimerasa (PCR) con el fin de determinar la presencia de 10 genes de resistencia contra aminoglicósidos [*ant(6)-Ia, ant(9)-Ia, ant(4′)-Ia, aph(3′)-IIIa, aph(2″)-Ib, aph(2″)Ic, aph(2″)-Id, aac(6′)-Ie-aph(2″)-Ia, y aac(6′)-Ii*]. Se detectaron 5 genes de resistencia a aminoglicósidos, los más frecuentes fueron el *aac(6′)-Ii,* y el *ant(6)-Ia,* detectados en *E. faecium*. Siete de los aislados de *E. faecalis* resistentes a gentamicina, kanamicina o estreptomicina fueron negativos por PCR a la presencia de los genes de resistencia evaluados, lo cual indica que posiblemente existen otros genes de resistencia. El análisis filogenético de los aislados demostró que los mismos eran genéticamente diferentes. Estos resultados indican que las cepas de enterococos aisladas presentes en pollos son diversas y contienen genes de resistencias contra aminoglicósidos potencialmente no identificados.

Key words: poultry, enterococci, gentamicin, kanamycin, streptomycin, resistance

Enterococci are a leading cause of nosocomial infections in humans and have been indicated in sporadic, but lethal, infections in poultry. These infections are caused by several enterococcal species that are commonly found in the intestines of the birds. *Enterococcus faecalis* has been indicated in pulmonary hypertension syndrome and amyloid athropathy in broilers, whereas *Enterococcus durans* and *Enterococcus hirae* were agents responsible for bacteremia, encephalomalacia, neu-

rologic disorders, and endocarditis in chickens (18,23,29,30).

In addition to their importance in disease, enterococci are also important because of their ability to harbor multiple antimicrobial resistances. The possibility of transfer of antimicrobial-resistant bacteria from animals to humans has created increased interest in antimicrobials that are used in both veterinary and human medicine. Introduced into clinical application in the mid-1990s, aminoglycosides are one class of antimicrobials that are used in both veterinary and human medicine for treatment of bacterial infections (17,22,26). Although limited in use in food-animal production as a result of their toxic nature, aminoglycosides can be used for treatment of severe infections, such as nonspecific enteritis in poultry (19,26,27).

Much is known about aminoglycoside resistance genes in human enterococci isolates, but a paucity of data exists on the prevalence of these genes in enterococci from other sources, particularly those of animal origin. In this study, the prevalence as well as the distribution of genes conferring resistance to gentamicin, kanamycin, and streptomycin in enterococci from poultry carcasses were investigated.

MATERIALS AND METHODS

Bacterial isolation and speciation. Enterococci used in this study represented a subset of enterococcal isolates collected from 1999 to 2000 as part of the veterinary surveillance branch of the National Antimicrobial Resistance Monitoring System (NARMS) at the USDA-ARS and USDA-FSIS in Athens, GA (15). Enterococci were isolated from poultry carcass rinsates collected from processing facilities in different regions of the United States. All media used in this study were purchased from Becton Dickinson (Sparks, MD). Portions of poultry rinsates were inoculated into BBL Enterococcosel broth and incubated for 24 hr at 37 C to enrich for enterococci. Presumptive positive cultures were transferred onto BBL Enterococcosel agar and incubated for 24 hr at 37 C. Presumptive enterococcal isolates were subcultured onto trypticase soy agar (TSA) containing 5% defibrinated sheep blood. Enterococcal species identification was performed using the BBL Crystal Kit (Becton Dickinson), according to manufacturer instructions.

Antimicrobial susceptibility testing. Minimum inhibitory concentrations (MICs, in μg/ml) for enterococci were determined by broth microdilution using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems Limited, Westlake, OH), according to the manufacturer directions. A customized panel of antimicrobials for the NARMS program was used, and it included gentamicin, kanamycin, and streptomycin. Results were interpreted according to NCCLS guidelines (21). *Enterococcus faecalis* ATCC 29212 was the positive control for determination of MIC.

Polymerase chain reaction (PCR) and sequencing of aminoglycoside resistance genes. Oligonucleotides were synthesized by Operon Technologies (Alameda, CA). The primers and cycling conditions used for detecting *aph(2″)-Ib* and *aph(2″)-Id* were as previously published (13). Multiplexing primers and cycling conditions for *aac(6′)-Ii, aac(6′)-Ie-aph(2″)-Ia, ant(4″)-Ia, ant(6)-Ia, ant(9)-Ia, aph(2″)-Ic,* and *aph(3″)-IIIa* were also as previously described (14). Positive controls for PCR were as follows: *E. faecium* SF11770 [*aph(2″)-Ib*] (13), *E. casseliflavus* SF11300 [*aph(2″)-Id*] (32), *E. faecium* G128E-1013 [*aac(6′)-Ii*] (this study), *E. faecalis* ATCC 49532 [*aac(6′)-Ie-aph(2″)-Ia*] (7), *E. gallinarum* 15N12-928 [*ant(4″)-Ia*] (this study), *E. faecalis* ATCC 49533 [*ant(6)-Ia*] (7), *Escherichia coli* NM554 (pAT28) [*ant(9)-Ia*] (31), *E. gallinarum* SF9117 [*aph(2″)-Ic*] (6), and *E. faecium* 10N-55-1023 [*aph(3″)-IIIa*] (this study). *Enterococcus faecium* G128E-1013 [*aac(6′)-Ii*], *E. gallinarum* 15N12-928 [*ant(4″)-Ia*], and *E. faecium* 10N-55-1023 [*aph(3″)-IIIa*] were all obtained from swine fecal samples and were verified by sequencing. PCR was performed in a PTC-200 thermal cycler (MJ Research, Waltham, MA). Whole-cell template for PCR was prepared by inoculating 100 μl of sterile deionized water with a single isolated bacterial colony. A reaction mixture (50 μl) consisting of 2 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase (Roche Molecular Biochemicals, Indianapolis, IN), 200 μM each dNTP, 50 pmol of each oligonucleotide, and 5 μl of whole-cell template were incubated at 95 C for 5 min. This initial denaturation step was followed by 30 PCR cycles of denaturation at 94 C for 1 min, annealing at 50 C for 1 min, and extension at 72 C for 1 min. Ten microliters of each PCR product were separated on a 1.5% agarose gel by electrophoresis at 90 V for 1 hr and were visualized by staining with ethidium bromide. PCR products were sequenced at the ARS Regional Sequencing Facility (Southeastern Poultry Research Laboratory, Athens, GA), and sequences were compared using NCBI-BLAST analysis (2).

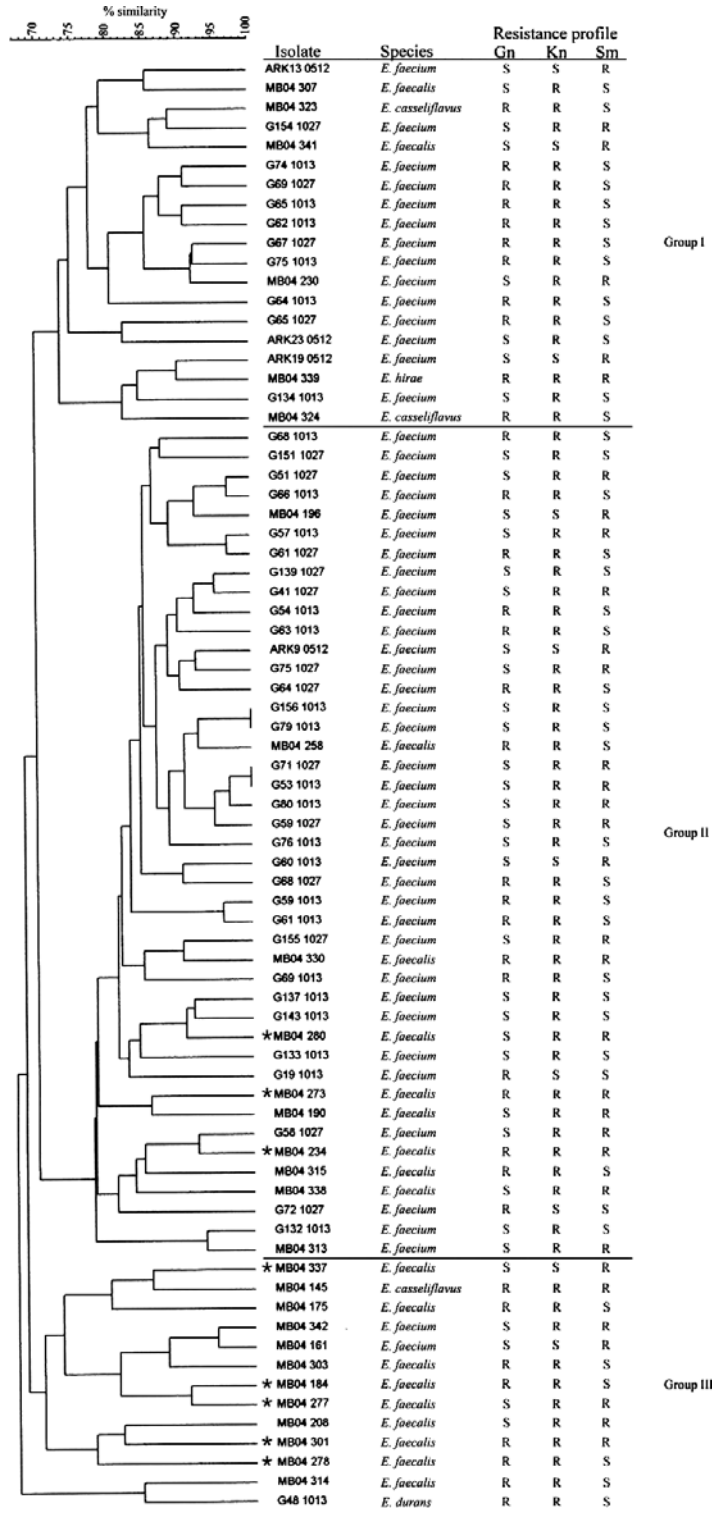
Pulsed-field gel electrophoresis (PFGE) and cluster analysis. PFGE using *Sma*I-digested DNA was performed as previously described (33). *Saccharomyces cerevisiae* chromosomes (BioWhittaker, Rockland, ME) were used as molecular standards for PFGE. Cluster analysis was determined with Bionumerics software (Applied Maths, Belgium) using Dice coefficient and the unweighted pair group method. Optimization settings for dendrograms were 8%, with a band tolerance of 2%–4% (1).

Table 1. Aminoglycoside resistance gene profiles in poultry enterococci.

Species	Antimicrobial MIC (μg/ml)			AME gene combination	No. isolates
	Gentamicin	Kanamycin	Streptomycin		
E. casseliflavus ($n = 3$)	>2048	>2048	>2048	*aac(6′)-Ie-aph(2″)-Ia–ant(6)-Ia–aph(3′)-IIIa*	2
	>2048	>2048	≤128	*aac(6′)-Ie–aph(2″)-Ia*	1
E. durans ($n = 1$)	>2048	>2048	≤128	Negative	1
E. faecalis ($n = 19$)	≤64	≤64	>2048	*ant(6)-Ia*	1
	≤64	2048	>2048	*ant(6)-Ia–aph(3′)-IIIa*	1
	≤64	>2048	>2048	*ant(6)-Ia–aph(3′)-IIIa*	2
	>2048	2048	≤128	*aac(6′)-Ie–aph(2″)-Ia*	1
	>2048	>2048	≤128	*aac(6′)-Ie–aph(2″)-Ia*	3
	>2048	>2048	≤128	*aac(6′)-Ie-aph(2″)-Ia–ant(6)-Ia–aph(3′)-IIIa*	1
	>2048	>2048	1024	*ant(6)-Ia–aph(3′)-IIIa*	1
	≤64	≤64	>2048	Negative	1
	≤64	512	≤128	Negative	1
	≤64	>2048	>2048	Negative	2
	>2048	>2048	≤128	Negative	2
	>2048	>2048	1024	Negative	1
	>2048	>2048	>2048	Negative	2
E. faecium ($n = 51$)	≤64	128	1024	*aac(6′)-Ii–ant(6)-Ia*	1
	≤64	256	1024	*aac(6′)-Ii–ant(6)-Ia*	1
	≤64	256	2048	*aac(6′)-Ii–ant(6)-Ia*	2
	≤64	256	>2048	*aac(6′)-Ii–ant(6)-Ia*	1
	≤64	256	>2048	*aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii–ant(6)-Ia*	1
	≤64	512	≤128	*aac(6′)-Ii*	1
	≤64	512	≤128	*aac(6′)-Ii–ant(6)-Ia*	7
	≤64	512	256	*aac(6′)-Ii*	1
	≤64	512	512	*aac(6′)-Ii–ant(6)-Ia*	1
	≤64	512	1024	*aac(6′)-Ii–ant(6)-Ia*	2
	≤64	512	2048	*aac(6′)-Ii–ant(6)-Ia*	1
	≤64	512	>2048	*aac(6′)-Ii*	1
	≤64	512	>2048	*aac(6′)-Ii–ant(6)-Ia*	9
	≤64	512	>2048	*aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii–ant(6)-Ia*	1
	≤64	1024	≤128	*aac(6′)-Ii*	1
	512	>2048	≤128	*aac(6′)-Ii*	1
	1024	>2048	≤128	*aac(6′)-Ii*	6
	1024	>2048	≤128	*aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii*	4
	2048	>2048	≤128	*aac(6′)-Ii*	1
	2048	>2048	≤128	*aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii*	1
	>2048	≤64	≤128	*aac(6′)-Ii*	1
	>2048	>2048	≤128	*aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii*	3
	>2048	>2048	≤128	*aac(6′)-Ii*	2
E. hirae ($n = 1$)	1024	>2048	≤128	*aac(6′)-Ie-aph(2″)-Ia–ant(6)-Ia–aph(3′)-IIIa*	1

→

Fig. 1. Dendrogram of high-level aminoglycoside-resistant enterococci from poultry carcass rinsates. Isolate, species, and resistance to gentamicin, kanamycin, and streptomycin are shown. Three groups (I, II, and III) were formed, with at least 71% overall similarity. Asterisks indicate aminoglycoside gene–negative *E. faecalis*. Levels of similarity were determined using Dice coefficient.


% similarity
70
75
80
85
90
95
100
Isolate
Species
Resistance profile
Gn
Kn
Sm
ARK13 0512 E. faecium S S R
MB04 307 E. faecalis S R S
MB04 323 E. casseliflavus R R S
G154 1027 E. faecium S R R
MB04 341 E. faecalis S S R
G74 1013 E. faecium R R S
G69 1027 E. faecium R R S
G65 1013 E. faecium R R S
G62 1013 E. faecium R R S
G67 1027 E. faecium R R S
G75 1013 E. faecium R R S
MB04 230 E. faecium S R R
G64 1013 E. faecium R R S
G65 1027 E. faecium R R S
ARK23 0512 E. faecium S R S
ARK19 0512 E. faecium S S R
MB04 339 E. hirae R R R
G134 1013 E. faecium S R S
MB04 324 E. casseliflavus R R S
G68 1013 E. faecium R R S
G151 1027 E. faecium S R S
G51 1027 E. faecium S R R
G66 1013 E. faecium R R S
MB04 196 E. faecium S S R
G57 1013 E. faecium S R R
G61 1027 E. faecium R R S
G139 1027 E. faecium S R S
G41 1027 E. faecium S R R
G54 1013 E. faecium R R S
G63 1013 E. faecium R R S
ARK9 0512 E. faecium S S R
G75 1027 E. faecium S R R
G64 1027 E. faecium R R S
G156 1013 E. faecium S R S
G79 1013 E. faecium S R S
MB04 258 E. faecalis R R S
G71 1027 E. faecium S R R
G53 1013 E. faecium S R R
G80 1013 E. faecium S R R
G59 1027 E. faecium S R R
G76 1013 E. faecium S R S
G60 1013 E. faecium S S R
G68 1027 E. faecium R R S
G59 1013 E. faecium R R S
G61 1013 E. faecium R R S
G155 1027 E. faecium S R R
MB04 330 E. faecalis R R R
G69 1013 E. faecium R R S
G137 1013 E. faecium S R S
G143 1013 E. faecium S R S
★MB04 280 E. faecalis S R R
G133 1013 E. faecium S R S
G19 1013 E. faecium R S S
★MB04 273 E. faecalis R R R
MB04 190 E. faecalis S R R
G58 1027 E. faecium S R R
★MB04 234 E. faecalis R R R
MB04 315 E. faecalis R R S
MB04 338 E. faecalis S R R
G72 1027 E. faecium R S S
G132 1013 E. faecium S R S
MB04 313 E. faecium S R R
★MB04 337 E. faecalis S S R
MB04 145 E. casseliflavus R R R
MB04 175 E. faecalis R R S
MB04 342 E. faecium S R R
MB04 161 E. faecium S S R
MB04 303 E. faecalis R R S
★MB04 184 E. faecalis R R S
★MB04 277 E. faecalis S R R
MB04 208 E. faecalis S R R
★MB04 301 E. faecalis R R R
★MB04 278 E. faecalis R R S
MB04 314 E. faecalis R R S
G48 1013 E. durans R R S
Group I
Group II
Group III

RESULTS AND DISCUSSION

Identification of poultry enterococcal species. One hundred and sixty-two enterococci were isolated from poultry carcass rinsates. Six species of enterococci were identified, including *E. avium*, *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, and *E. hirae*. The predominant species identified were *E. faecium* ($n = 105$), *E. faecalis* ($n = 40$), and *E. durans* ($n = 8$). Although isolates in this study originated from chicken carcasses, results are consistent with those from another study in which *E. faecium* was the predominant species isolated from intestinal sites of 4-wk-old chickens (9). Conversely, in 1-day-old chicks, *E. faecalis* is the most common species, whereas *E. cecorum* is prevalent in older birds.

Prevalence of gentamicin, kanamycin, and streptomycin resistance. For enterococci, break points for high-level gentamicin resistance (HLG^r) and high-level streptomycin resistance (HLS^r) are $\geq$500 μg/ml and $\geq$1000 μg/ml, respectively. In this study, MIC for high-level kanamycin resistance (HLK^r) was also $\geq$500 μg/ml. Seventy-five isolates (46%) exhibited high-level resistance to at least one aminoglycoside (gentamicin, kanamycin, or streptomycin). The majority of resistant isolates (89%) were resistant to kanamycin ($n = 67$), whereas 49% and 41% of resistant isolates were resistant to gentamicin ($n = 37$) and streptomycin ($n = 31$), respectively. The prevalence of resistance greatly diminished when the overall samples were considered. Twenty-three percent, 41%, and 19% of poultry enterococci exhibited high-level resistance to gentamicin, kanamycin, and streptomycin, respectively. These figures are higher than those from a previous report of 0%–3% gentamicin resistance but are within the reported 12%–26% streptomycin resistance range (3). Prevalent kanamycin resistance has also been documented in enterococci from environmental samples (24).

For all three antimicrobials, *E. faecium* ($n = 51$) and *E. faecalis* ($n = 19$) were the species with the highest number of resistant isolates. Fifty-six percent and 29% of HLG^r isolates, 67% and 25% of HLK^r isolates, and 58% and 35% of HLS^r isolates were *E. faecium* and *E. faecalis*, respectively. Although *E. faecium* accounted for higher percentages of resistant isolates, 47% of all *E. faecalis* isolates were resistant to at least one aminoglycoside, compared to 48% of *E. faecium* isolates. Two *E. casseliflavus* and three *E. faecalis* isolates were multiresistant to all three antimicrobials (Table 1). No resistance was observed in *E. avium* isolated from poultry.

Prevalence of AME genes. In order to discern which AME genes might be responsible for mediating resistance, isolates were tested by PCR using primers to 10 AME genes. The challenge of performing PCR on the number of isolates in this study was minimized using a recently developed multiplex PCR for AME genes (14). Of the 10 genes examined, five AME genes [*ant(6)-Ia, aph(3′)-IIIa, aac(6′)-Ie-aph(2″)-Ia,* and *aac(6′)-Ii*] were identified (Table 1). Because of the number of *E. faecium* isolated, *aac(6′)-Ii* ($n = 52$) was the most frequently amplified product, followed by *ant(6)-Ia* ($n = 35$). The *aac(6′)-Ii* gene, located on the chromosome, is unique to *E. faecium*. The prevalence of *ant(6)-Ia* conferring resistance to streptomycin was not unexpected, since this gene has been reported to be widespread in enterococci from humans (12). The low number of isolates identified containing the bifunctional gene *aac(6′)-Ie-aph(2″)-Ia* ($n = 19$) in this study was surprising. This gene has been found on plasmids in *Enterococcus* and *Staphylococcus aureus* and mediates resistance to all clinically important aminoglycosides (11,25). The scarcity of *aac(6′)-Ie-aph(2″)-Ia* in these isolates indicates that it is not as common in enterococci from poultry as in enterococci from other sources. Conversely, *ant(4′)-Ia* and *ant(9)-Ia,* both originating from *S. aureus*, have not been commonly associated with AME resistance in enterococci (16,20). Furthermore, *ant(9)-Ia,* which mediates resistance to spectinomycin, was only just recently reported in strains of *E. faecalis* and *E. faecium* in Japan (14). Three genes [*aph(2″)-Ib, aph(2″)-Ic,* and *aph(2″)-Id*] may not have been detected in the isolates because, having only recently been described in the enterococci, they may not be as widely disseminated among these bacteria as other AME genes (6,13,32). Nine resistant *E. faecalis* isolates and one resistant *E. durans* isolate were negative for all of the genes tested, indicating alternative mechanisms of resistance, such as ribosomal mutations for streptomycin resistance (10,28).

Surveys of AME genes have revealed that strains of enterococci can harbor multiple AME genes (4,5,14,34). Six different combinations of AME genes were distributed among five species in this study (Table 1). Three of the six combinations consisted of three or more genes, including the bifunctional *aac(6′)-Ie-aph(2″)-Ia* (Table 1). The least and most prevalent combination, *aac(6′)-Ie-aph(2″)-Ia–aac(6′)-Ii–ant(6)-Ia* and *aac(6′)-Ii–ant(6)-Ia,*

respectively, were found in isolates of *E. faecium* only. One combination identified in multiple enterococcal species was *aac(6′)-Ie-aph(2″)-Ia–ant(6)-Ia–aph(3′)-IIIa,* detected in *E. casseliflavus*, *E. faecalis*, and *E. hirae* (Table 1). Because synergy between the aminoglycosides and cell-wall–inhibiting antimicrobials has been previously described and is important clinically, the combinations of genes conferring resistance to aminoglycoside antimicrobials in enterococci from poultry are of great concern (8).

Association of aminoglycoside resistance and AME genes. Enterococcal isolates resistant to gentamicin, kanamycin, or streptomycin and a combination of the three were examined for their AME gene content to determine the relationship between the resistance profile and AME gene. For gentamicin resistance, a PCR product was not detected in 17 resistant isolates (Table 1). Alternatively, two isolates that were gentamicin susceptible contained genes [*aac(6′)-Ie-aph(2″)-Ia*] conferring gentamicin resistance. The same phenomenon was observed for kanamycin and streptomycin. For kanamycin resistance, eight isolates were resistant, but no resistance gene was detected. Six isolates containing a kanamycin resistance gene [*aac(6′)-Ii*] displayed low-level (MIC 128–256 μg/ml) resistance, while one isolate was considered susceptible (MIC $\leq$ 64 μg/ml) (Table 1). In addition, seven isolates were streptomycin resistant but did not contain a streptomycin resistance gene, and nine isolates contained *ant(6)-Ia* but were not streptomycin resistant.

Several reasons for these observations exist. To begin with, five, eight, and six isolates that were gentamicin, kanamycin, or streptomycin resistant but that were negative for resistance genes were *E. faecalis* from the same source. These isolates could represent a cluster containing unrecognized aminoglycoside resistance genes or differences in the PCR priming region. This could also be true of other resistant isolates for which a resistance gene was not amplified. Streptomycin-resistant isolates without a detected resistance gene are most likely the result of mutations in the ribosomes. But more complex is the detection of a resistance gene in susceptible isolates. This could be the result of mutations in the gene leading to a nonexpressed or inactive gene. Studies on those isolates are ongoing.

Genetic relatedness of resistant isolates. Phylogenetic analysis was performed in order to identify any genetic relatedness among the isolates. Although clusters were examined by isolate, species, and aminoglycoside resistance profile, no distinct clustering could be defined based solely upon these criteria (Fig. 1). Groups were defined as clusters having at least 70% similarity.

From the analysis, three major clusters were evident. Group I contained 19 isolates of various species originating from different regions sharing at least 73% similarity (Fig. 1). Group II was the largest cluster, consisting of 57% ($n = 43$) of the isolates, mostly *E. faecium*. Whereas isolates in group II had the highest similarity (78%), group III isolates had the lowest similarity, at 71% similarity between the isolates. Group III was also the smallest cluster, with 13 isolates. This group contained five of the nine *E. faecalis* that were negative for all aminoglycoside resistance genes tested (Fig. 1). Three of the negative *E. faecalis* isolates were located on two minor clusters in group II. Although the isolates were in another group, they were spatially very near to group III, indicating that they were closely related to each other. The remaining aminoglycoside-negative *E. faecalis* isolate was located in group I. Surprisingly, only two of the isolates were identical to other isolates, indicating that the isolates in this study were genetically distinct and did not originate from the same source (Fig. 1).

Antimicrobial resistance continues to create difficulties in treatment of diseases. Even though enterococci are not considered pathogenic bacteria, they are causative agents of disease and are often resistant to the antimicrobials used for treatment. In addition, they are capable of transferring resistances to other bacteria, aiding in persistence of antimicrobial resistance. Because of the unknown role enterococci may have in diseases of poultry, future studies will continue to investigate enterococci from this source.

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